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Species cross-reaction of α-fetoproteins and break-down of the tolerance to α-fetoprotein by immunization with heterologous α-fetoprotein

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Rabbit antibody raised against human α-fetoprotein (AFP) by injecting pure AFP cross-reacted with rabbit AFP by the Ouchterlony test. Similar phenomenon was observed with horse antiserum prepared by immunization with human AFP and the horse antiserum cross-reacted with horse AFP[1]. These findings are assumed to be analogous to the experiments of Dr. Weigle who stated that rabbits were unresponsive to the injection of homologous thyroglobulin but, when injected with immunologically cross-reacting bovine thyroglobulin, they produced antibodies and some of the antibodies cross-reacted with rabbit thyroglobulin and autoimmune thyroiditis was caused by the autoantibodies[2].

This paper describes studies concerning the following 4 problems on AFP.

1) *Immunological relationship of human and animal AFP*

Immunological cross-reactions of anti-human AFP and several animal AFP have been reported by Dr. Gitlin and the authors using rabbit[3,4] or horse antiserum[4].

In the present study, chickens were immunized with human AFP. Chickens are a more remote species than rabbits or horses and they should recognize a greater number of determinants on human AFP. No reaction could be observed between the rabbit or horse antiserum to human AFP and newborn chicken serum.

Chickens were given purified human AFP subcutaneously with Freund's complete adjuvant 5 times at intervals of 7–15 days and the amount of antigen per injection was 1 mg. The antisera were absorbed with horse and human serum to remove trace amounts of antibodies to human and horse γ-globulin. Antibody concentrate was prepared from the pooled antiserum by Na_2SO_4 precipitation[5] and the protein concentration was 10%. The Ouchterlony test was performed in 8% NaCl.

The antibody concentrate did not react with sera of adult human, dog,

cat, pig, rabbit, horse, sheep, goat, cow, rat and mouse but formed specific immune precipitates with human AFP and newborn sera of the above ten animal species. AFP of dog, cat, pig were strongly cross-reactive with the antibody but the reactions with rat and mouse AFP were weak. AFP of rabbit, horse, sheep, goat and cow were moderately reactive. All reactions of the animal AFP showed partial identities to human AFP.

The antibodies cross-reacting with dog, rabbit, rat and mouse AFP were quantitated by the Mancini method using purified AFP preparations and agar gel incorporated with chicken antibody. In the Mancini method, the size of the precipitin ring is proportional to the amount of antigen and

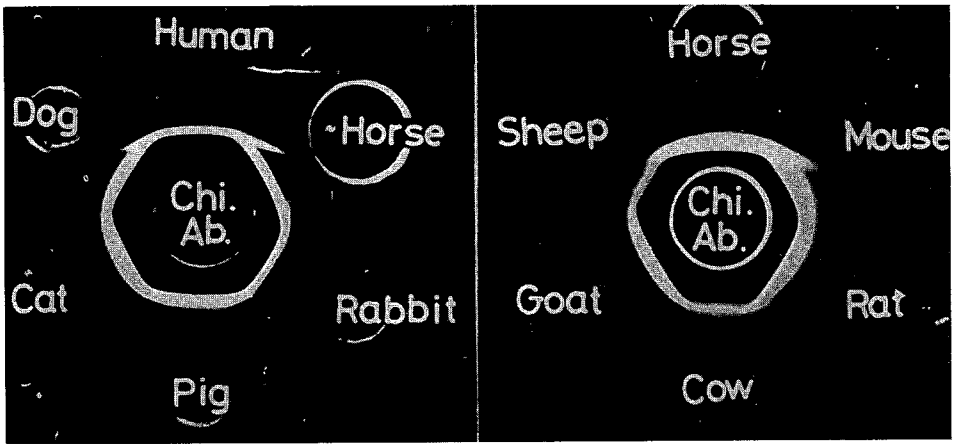
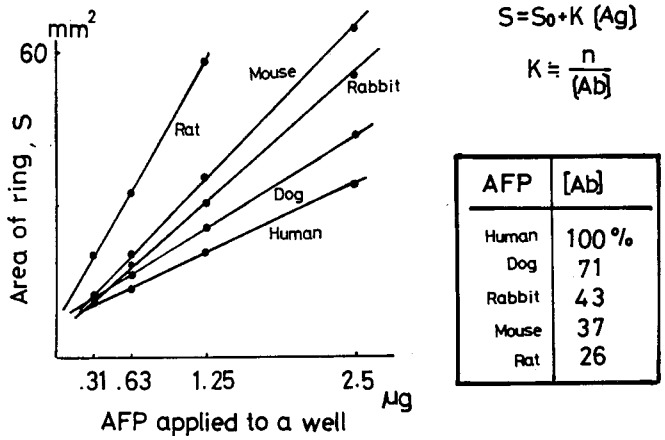


Fig. 1. Immunological cross-reaction of chicken antibody to human AFP with animal AFP



$$S = S_0 + K[Ag]$$

$$K \fallingdotseq \frac{n}{[Ab]}$$

AFP	[Ab]
Human	100 %
Dog	71
Rabbit	43
Mouse	37
Rat	26

Fig. 2. Quantitation of the cross-reacting antibodies with dog, rabbit, rat and mouse AFP

the slope is inversely related to the antibody concentration in gels[6]. The rate of the cross-reaction was calculated from the slope. Seventy-one% of antibody to human AFP was reactive to dog AFP, 43% to rabbit AFP, 37% to mouse AFP and 26% to rat AFP.

As described above, a very close immunological relationship of human and animal AFP was clearly demonstrated with chicken antibody.

2) *Responses to the injection of homologous AFP*

AFP which is one of the main constituents of serum proteins during fetal periods is present only in trace amounts in adults. Thus it is of interest to determine whether adult animals are immunologically responsive to the administration of homologous AFP or not.

Rabbits, dogs and rats received injections of sera of newborn homologous species by a similar method used to prepare chicken antiserum. The amount of newborn serum per injection given to rabbits or dogs was 0.5 ml and rats were given half a dose.

The antisera were tested for antibodies by reacting with sera from homologous newborns but no antibody to AFP could be demonstrated by the Ouchterlony test indicating immunological unresponsiveness of these adult animals to homologous AFP.

3) *Responses to the injection of human AFP*

Rabbits, horses, rats and a dog were immunized with human AFP. The antisera obtained from 3 rabbits, 5 rats, 3 horses and a dog were tested for their specificities to AFP by reacting them with the sera of hepatoma patients and healthy adults and were found to be satisfactory. The sera obtained from newborn and adult rabbits, rats, horses and dogs were reacted with these antisera. All antisera failed to form specific im-

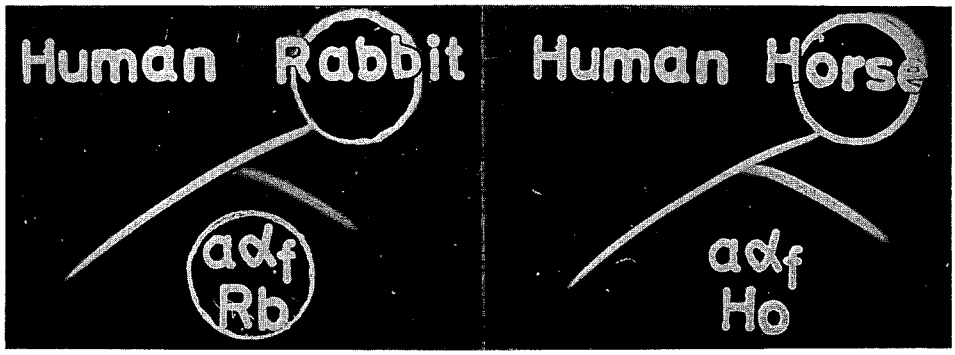


Fig. 3. Reactions of rabbit anti-human AFP (aαfRb) and horse anti-human AFP (aαfHo) with human, rabbit and horse AFP

mune precipitates with adult sera but some of them reacted with the newborn sera.

Rabbit and horse antisera reacted with the sera of newborn rabbits and newborn colts, respectively.

A similar reaction was observed with rat antibody fraction which was 5 times more potent than the original serum.

Dog which AFP is the closest to that of human subjects showed the poorest response to the injection of human AFP and the reaction with homologous AFP could not be demonstrated.

As described above, the injection of human AFP provoked antibodies to homologous AFP in rabbits, rats and horses.

The liver of these animals were examined microscopically but no histopathological change was demonstrable.

4) *Effects of maternal antibody against AFP to fetuses*

Antibody to homologous AFP was raised in female rabbits by injecting human AFP. Rabbits were bred resulting in pregnancy to examine the effects of the maternal antibody to fetuses.

No apparent abnormality was observed in newborn rabbits, macroscopically. The sera of newborns and mothers were tested for anti-human AFP, anti-rabbit AFP and rabbit AFP. Newborn rabbit sera gave positive tests for antibody to human AFP. This finding suggests the transfer of maternal antibody including anti-rabbit AFP to fetuses during pregnancy. Anti-rabbit AFP could be detected in early pregnant rabbits but not in post-partal rabbits.

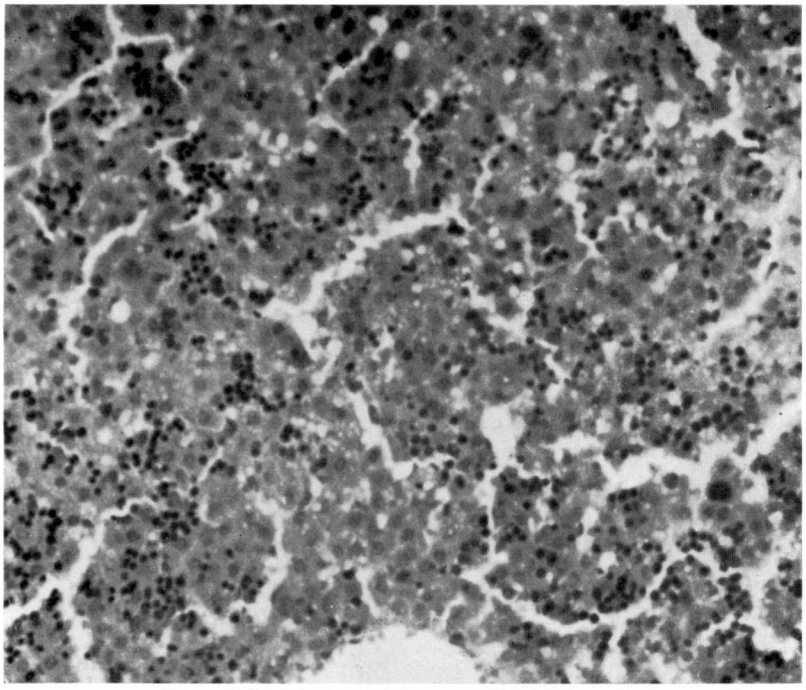
In newborns, rabbit AFP was present instead of anti rabbit AFP.

The histological features of liver, kidney and spleen of the newborn were examined. The liver cells were severely damaged as shown in Fig. 4.

This type of change was observed in all newborns from 5 different litters although the degree was slightly different. By Sudan III staining, fatty degeneration of the liver cells was demonstrated. These changes were limited in the liver and no marked histological changes were observed in the spleen or kidney.

The liver cells are the main site of AFP production and the histological changes found in the cells could be explained to be caused by maternal anti-rabbit AFP transfered to fetuses. Parts of these studies are previously reported[1].

control



experiment

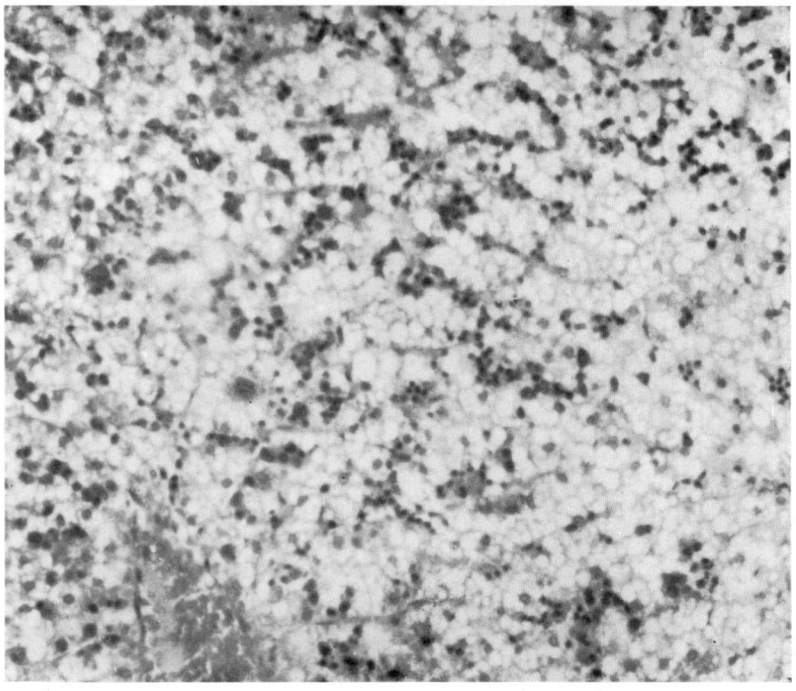


Fig. 4. Histological features of the newborn rabbit liver

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